Jilin-China aims to develop a novel cancer therapywith Bifidobacterium andwe have constructeda partfor them successfully.We added mRFP after the HU promoter to visualize its function.

1.Amplification of target fragments

In our project, in order to be able to finally amplify the target fragments.we try the following PCR systems and the procedure settings

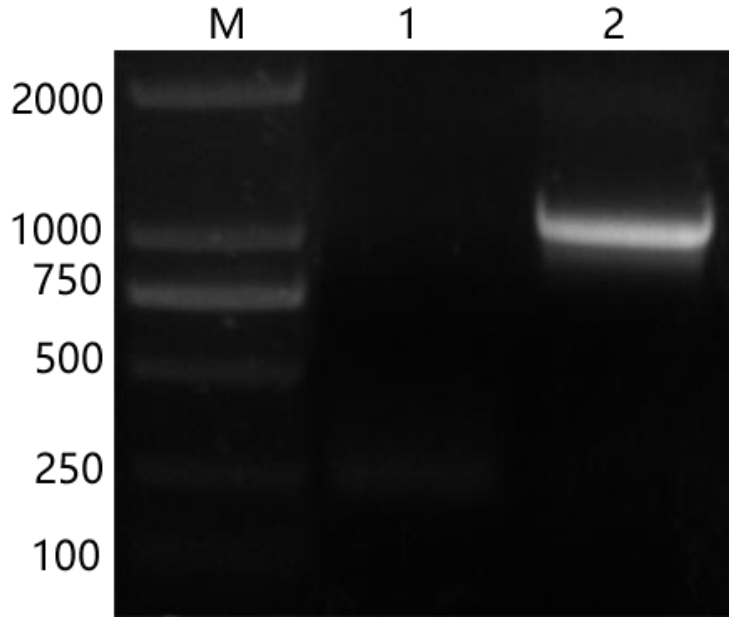
KOD amplification system

Component	50ul Reaction
10× KOD buffer	5ul
2mM dNTPs	5ul
25mM $MgSO_4$	3ul
10uM forward primer	1ul
10uM Reverse primer	1ul
Temp	1ul(1ng/l)
KOD enzyme	1ul
ddH_2O	33ul

KOD PCR program

Segment	Cycles	Temperature	Time
Initial denaturation	1	94° C	3min
Denaturation	38	94° C	30s
Renaturation	38	56° C	30s
Prolongation	38	68° C	30s/kb
Terminal prolongation	1	68° C	3min
heat preservation	1	12° C	10min

Result:



2.Fusion

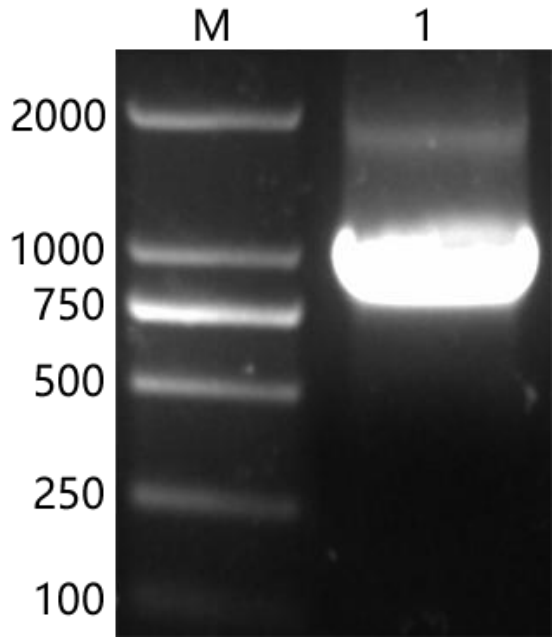
System

Component	50l Reaction
10× KOD buffer	5l
2mM dNTPs	5l
25mM $MgSO_4$	3l
Forward primer	1l
Reverse primer	1l
fragment 1	the moles rate of fragment1 to fragment2 is 1:1
fragment 2	
KOD enzyme	1l
ddH_2O	to 50l

Program

Segment	Cycles	Temperature	Time
Initial denaturation	1	94	3min
Denaturation	38	94	30s
Renaturation	38	56	30s
Prolongation	38	68	30s/kb
Terminal prolongation	1	68	3min
heat preservation	1	12	10min

Result:



3.Colony PCR

MasterMix Preparation

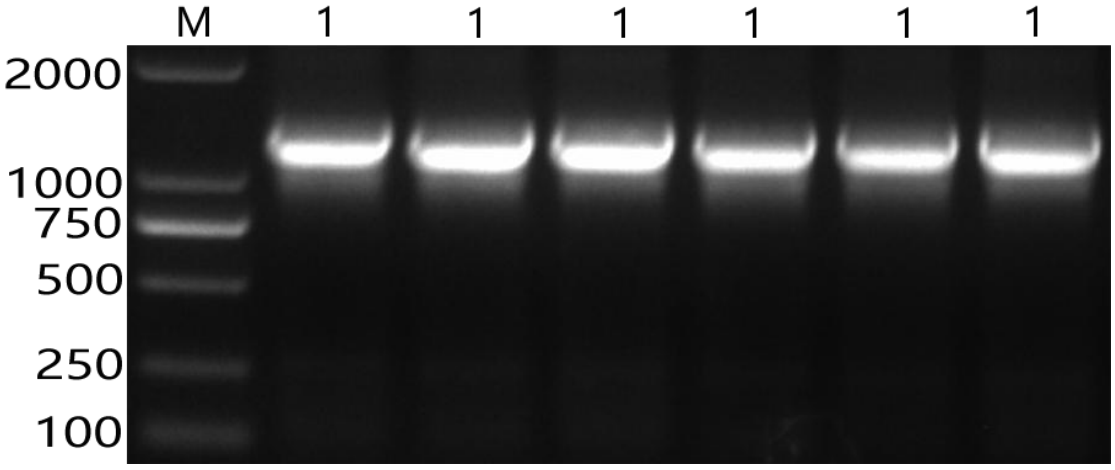
Component	25ul Reaction
10×buffer	2.5ul

10mM dNTP	0.5ul
10uM Primer 1	0.5ul
10uM Primer 2	0.5ul
Temp	5ul
Taq enzyme	0.4ul
ddH_2O	15.6ul

PCR Program Setting

Segment	Cycles	Temperature	Time
Initial denaturation	1	95° C	5min
Denaturation	38	94° C	30s
Renaturation	38	56° C	30s
Prolongation	38	72° C	40s/kb
Terminal prolongation	1	72° C	5min
heat preservation	1	12° C	10min

Result:



Conclusion

We characterized the part successfully.